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Architecture and Dynamics of the Transcription Factor Network that Regulates B-to-Plasma Cell Differentiation

Kazuhiko Igarashi*, Kyoko Ochiai and Akihiko Muto

Department of Biochemistry, Tohoku University Graduate School of Medicine, Seiryo-machi 2-1, Sendai 980-8575, Japan

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Upon antigen stimulation, B lymphoid cells undergo terminal differentiation into antibody-secreting plasma cells. This process accompanies drastic changes in cell functions such as a loss of B-cell identity, induction of secretory apparatus, and an extremely increased transcription of antibody genes. These changes are the result of re-wiring of a transcription factor network in B and plasma cells. While the transcription repressor Blimp-1 induces plasma cell differentiation, another repressor Bach2 has emerged as a negative regulator of Blimp-1 in B cells. These two transcription factors, together with other several factors, appear to constitute a main transcriptional regulatory network for the terminal differentiation process of plasma cells from B cells.

Key words: Bach2, Blimp-1, class switch recombination, plasma cells, transcription factor.

Abbreviations: AID, activation-induced cytidine deaminase; Bach2, BTB and CNC homology 2; Bcl6, B-cell lymphoma 6; Blimp-1, B lymphocyte-induced maturation protein 1; bZip, basic region-leucine zipper; CLS, cytoplasmic localization signal; CSR, class switch recombination; GC, germinal centre; Ig, immunoglobulin; IRF-4, interferon regulatory factor 4; LPS, lipopolysaccharide; MARE, Maf-recognition element; MITF, microphthalmia-associated transcription factor; Pax5, paired box gene 5; SHM, somatic hypermutation; XBP-1, X-box binding protein 1.

The process of cell differentiation is usually composed of multiple steps, each of which is characterized by a distinct gene expression pattern. Because the gene expression patterns are defined primarily by transcription factors that are active in particular differentiation stages, the transition from one stage to the next may thus involve the reprogramming of the transcription factor networks. B lymphoid cells provide an excellent model system to understand the transcription factor networks that regulate cell differentiation, because each step of B-cell differentiation is well defined and can be monitored and accessed using various molecular markers. Mature B cells in peripheral lymphoid tissue initiate the terminal differentiation process into plasma cells in response to the antigen cue. The results of recent studies on several critical transcription factors allow us to construct a transcription factor network which stably maintains cells at the undifferentiated mature B-cell stage or the fully differentiated plasma cell stage (*i.e.* bi-stable). This network is nonetheless dynamic in its responsibility to antigens.

OUTLINE AND ISSUES OF B-CELL DIFFERENTIATION

B cells develop from the haematopoietic stem cells in the bone marrow and continue to undergo maturation in the spleen in mice (*1*). In antibody responses, B cells are induced by the antigen or polyclonal stimulators to proliferate and differentiate into the antibody-secreting plasma cells. As such, the terminal differentiation of B cells into plasma cells can be viewed as an instructive process. There are multiple alternate cell fates through which antigen-activated B cells can follow. One is to become plasma cells that secrete immunoglobulin M (IgM). The second fate is to become plasma cells that secrete isotype immunoglobulins, such as IgG. Along this line, B cells undergo class switch recombination (CSR), in order to modify the immunoglobulin heavy chain (IgH) gene (*2*). This process results in the modification of the effector functions of the antibodies. Somatic hypermutation (SHM) is also introduced into the Ig genes in order to increase antigen-binding affinity. Activation-induced cytidine deaminase (AID) is essential for these two mutagenic events (*3*). Both CSR and SHM occur in the B cells that proliferate in the germinal centre (GC) and which express AID. The third fate for such antigen-activated B cells is to follow CSR and SHM to become memory B cells, rather than plasma cells, in order to prepare against future encounters with the antigen (*4*). It should be noted that both CSR and SHM require a considerable amount of time and cell proliferation after the antigen encounter, whereas the IgM-producing plasma cells are generated rapidly in response to the antigen. The issue is then, how these options are registered in mature B cells and how they are selected upon activation. The events associated with the B-cell activation are not fully cell-autonomous and are regulated in part by the interaction with other cells, including T cells, and the microenvironment (*5*). In response to the antigen, B cells move through multiple

*To whom correspondence should be addressed. Tel: 81-22-717-7595, Fax: 81-22-717-7598,
E-mail: igarak@mail.tains.tohoku.ac.jp

microenvironments on their way to becoming plasma cells. However, this aspect of the microenvironments is not the scope of this review. Two major issues, which are both cell-intrinsic, are addressed in this review. One is the architecture of the transcription factor network in B and plasma cells, and the nature of its rewiring which is associated with the transition from B cells to plasma cells. The second is how the alternate fates (*e.g*. to switch or not to switch) are selected upon the terminal differentiation process. The interaction of these two events (*i.e*. the rewiring of the regulatory network and fate selection) will also be discussed.

TRANSCRIPTION FACTORS THAT REGULATE B-TO-PLASMA CELL DIFFERENTIATION

It is reasonable to presume that the processes of B-cell activation and plasma cell differentiation involve the rewiring and consolidation of the transcription factor networks (*6*, *7*). Several genetic and biochemical studies have examined the critical transcription factors. Some of these studies are herein briefly described and their regulatory relationships are summarized.

Pax5—Pax5 is a paired homeodomain protein that is expressed in B cells and the nervous system (*8*). In B cells, the expression of Pax5 initiates in the pro-B cells, continues in the mature B cells, and is silenced upon plasma cell differentiation. Pax5 is required for the rearrangement of the immunoglobulin genes (*9*). While DJ gene segments are rearranged normally in the *Pax5*$^{-/-}$ B cells, the rearrangement of the Vh-gene segments is severely impaired. Pax5 functions as both an activator and a repressor (*10*). Pax5 activates the genes that are involved in the functions of the pre-B-cell receptor (pre-BCR) and the B-cell receptor (BCR), and represses the genes that are inappropriate with the B-cell lineage (*11*). The *Pax5*$^{-/-}$ pro-B cells show an abnormal plasticity and can differentiate *in vitro*, after stimulation with the appropriate cytokines, into macrophages, osteoclasts, dendritic cells, granulocytes or natural killer cells (*12*). Furthermore, after transfer to Rag2-deficient mice, which lack B cells and T cells, the *Pax5*$^{-/-}$ pro-B cells provide long-term reconstitution of the thymus and generate mature T cells (*13*). In addition to the silencing of the lineage-inappropriate genes, Pax5 also represses the genes required for plasma cells in the mature B cells (*14*, *15*). When Pax5 is deleted in DT40 B cells, the expression of both Blimp-1 and XBP-1, two critical plasma cell transcription factors (see below), is strongly upregulated (*16*). During the normal differentiation process, the downregulation of Pax5 in stimulated B cells may promote the plasma cell differentiation of B cells.

Bach2—The basic region-leucine zipper (bZip) factor BTB and CNC homology 2 (Bach2) functions as a transcription repressor (*17*). It possesses BTB/POZ domain and bZip domain. Bach2 forms heterodimers with the small Maf proteins (MafK, MafG and MafF) through the leucine zipper, then binds to the Maf-recognition elements (MARE) (Fig. 1). Bach2 is abundantly expressed in both B cells and neurons (*18*, *19*). In the B-cell compartment, Bach2 is expressed from the pro-B cell to the mature B-cell stages but is absent in the plasmacytic cell lines. Its expression is also silenced in the primary

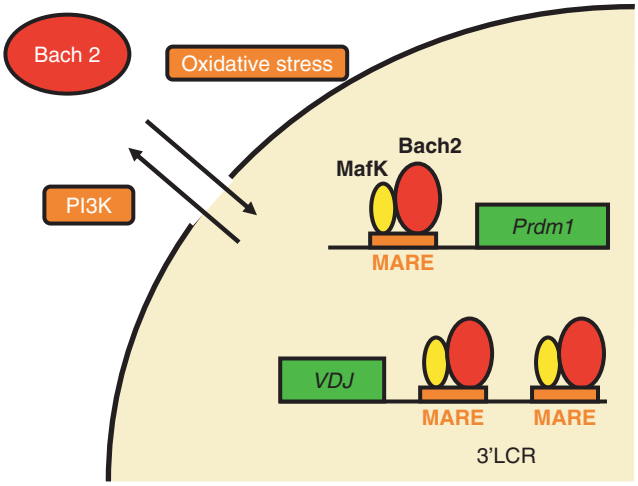

Fig. 1. **Subcellular localization of Bach2 protein.** Bach2 regulates the enhancer activity of the IgH3' LCR and Blimp-1 gene by binding to MAREs in these genes. Bach2 possesses a nuclear localization signal (NLS) in the bZip domain and a nuclear export signal (NES) at its C-terminus. Bach2 accumulates in the nuclei under oxidative stress, causing apoptosis of the leukaemia cells. The phosphorylation of Bach2 via the PI3K pathway induces Bach2 accumulation in the cytoplasm. Since B-cell differentiation is regulated by various receptors, including the B-cell receptor (BCR), Bach2 may function in response to these signalling pathways.

plasma cells (our unpublished observation). The genetic ablation of *Bach2* in mice severely abrogates both the T-cell-independent and T-cell-dependent CSR and SHM of the immunoglobulin genes (*20*). In contrast, the production of IgM is apparently not defective in the absence of Bach2. These phenotypes indicate that Bach2 is somehow required for CSR and SHM, while it is dispensable for the generation of IgM-producing plasma cells. Bach2 is the only transcription factor whose genetic ablation is thus far known to uncouple CSR/SHM and plasma cell differentiation. A straightforward prediction would be that Bach2 regulates the proper expression of the genes which are required either directly or indirectly for these events. Indeed, the expression of AID is severely reduced in Bach2-deficient B cells. Interestingly, the expression of Blimp-1 and XBP-1, which are both essential for plasmacytic differentiation, is upregulated in Bach2-deficient B cells. Bach2 represses the expression of Blimp-1 by binding to MARE on the Blimp-1 gene (*Prdm1*) (*21*). Because Blimp-1 represses the AID expression (*22*), the reduced expression of AID in the Bach2-deficient B cells may be due to the overexpression of Blimp-1. Bach2 inhibits the enhancer activity of the IgH 3′ locus control region (LCR) by binding to its MARE (*18*). Bach2 may inhibit the full activation of IgH before the terminal differentiation.

Two mechanisms are known to regulate the activity of Bach2 (Fig. 1). Bach2 possesses a none-typical nuclear export signal (cytoplasmic localization signal, CLS) at its C-terminus (*23*). The oxidative stress inhibits CLS activity, thus resulting in the nuclear accumulation of Bach2 (*23–25*). In B cells, the PI3K pathway stimulates the phosphorylation of Bach2 at one serine residue (Serine-521 in human BACH2) and enhances its

cytoplasmic accumulation (*26*). Bach2 is also regulated by SUMOylation, which enhances the accumulation of Bach2 around the PML nuclear bodies (*25*). The silencing mediator of retinoid and thyroid receptor (SMRT) and histone deacetylase 4 (HDAC4) interact with Bach2, enhancing the Bach2 localization around the PML nuclear bodies (*27*). How these regulations affect the function of Bach2 during B-cell differentiation requires further studies.

Bcl6—B-cell lymphoma 6 (Bcl6) is expressed at high levels in the GC B cells and is essential for the formation of GC, in which SHM occurs, for the production of high affinity immunoglobulin (*28–31*). Bcl6 possesses the BTB/POZ domain in the NH2-terminal region, and the Krüppel-type zinc finger motifs in the COOH-terminal region (*32*). Bcl6 functions as a transcriptional repressor by binding several co-repressors. The BTB/POZ domain of Bcl6 binds to the SMRT directly (*33*) and recruits HDACs (*34*, *35*). Bcl6 also interacts with MTA3, which then recruits the Mi-2/NuRD co-repressor complex (*36*).

During the B-cell differentiation, Bcl6 has the critical function of repressing the Blimp-1 gene. Both AP-1 and STAT3 are supposed to activate the Blimp-1 gene expression. Bcl6 represses the Blimp-1 gene by inhibiting the DNA binding of AP-1 through protein interaction (*37*) and competing with STAT3 for the binding sites (*38*). Bcl6 also represses the Blimp-1 gene directly through its binding site in the intron 5 of the Blimp-1 gene (*39*). In *Bcl6*$^{-/-}$ mice, the formation of GC is defective (*28–30*). Although Bcl6-defecient B cells can differentiate into IgM and IgG1 memory B cells, these have no somatic hypermutation in the *V*-heavy gene (*31*). These observations suggest that Bcl6 is essential for the formation of GC, and that it is required for somatic hypermutation (*31*). However, whether or not the expression of AID is affected by the absence of Bcl6 has not yet been examined. Considering the fact that CSR occurs in the *Bcl6*$^{-/-}$ B cells, Bcl6 may be required for SHM in an AID-independent pathway. It is noteworthy that, while Bach2 deficiency causes severe defects in CSR, Bcl6 deficiency allows for substantial levels of CSR.

Bcl6 also represses the tumour suppressor p53 gene (*40*) and the p21 gene by binding to Miz-1 (*41*). These functions of Bcl6 are probably essential for rescuing B cells from apoptosis, which may otherwise be induced in response to the DNA breaks during CSR and SHM in the GC. As B cells differentiate into plasma cells, the protein level of Bcl6 is rapidly diminished by the ubiquitin/proteasome pathway in response to B-cell receptor signalling (*42*). The expression of Bcl6 is repressed by Blimp-1 (*22*). This regulation may play a role in terminating the germinal center reactions upon the terminal differentiation into plasma cells.

MITF—The microphthalmia-associated transcription factor (MITF) is a basic-helix-loop-helix–leucine zipper protein which was discovered as the responsible gene of microphthalmos (*43*). MITF binds to the E-boxes as a homodimer. It has been reported that MITF plays an important role in the development of various types of cells, including melanocytes, retinal pigment epithelial cells, mast cells, natural killer cells and osteoclasts (*44–46*). In the B-cell compartment, MITF is highly expressed in the naïve B cells and is rapidly diminished in the activated B cells. One of the crucial functions of MITF in the B cells is to inhibit the expression of IRF-4 and thus to antagonize the process of terminal differentiation into plasma cells (*47*). The MITF-deficient B cells undergo a spontaneous differentiation into plasma cells, thus resulting in higher levels of serum IgM.

Blimp-1—B lymphocyte-induced maturation protein 1 (Blimp-1) carries Zn fingers for DNA binding and a SET domain, which may play a role in transcription repression (*48*). Among the several transcription factors known to regulate the differentiation of B cells into plasma cells, Blimp-1 appears to be the key regulator whose gene serves as a hub in the gene network for plasma cell differentiation (*22*, *49*). An overexpression of Blimp-1 induces the mature B cells to differentiate into plasma cells (*48*, *50*). On the other hand, the Blimp-1-deficient B cells fail to become either plasma cells or memory B cells (*51*). Since Blimp-1 downregulates a large number of the genes required for germinal center formation and/or activated B cells including Pax5 and Bcl6, Blimp-1 may therefore promote plasma cell differentiation by cancelling the B-cell identities (*22*, *49*, 52). Blimp-1 also represses *c-Myc* and inhibits cell proliferation (*53*), thus facilitating the cell cycle arrest associated with the terminal differentiation. Based on these observations, Blimp-1 has therefore been suggested as a master regulator of plasma cell differentiation.

The Blimp-1 expression is high in plasma cells but either low or absent in B cells (*54*), thus indicating that its expression is strictly regulated during plasma cell differentiation. As summarized above, Bach2 and Bcl6 are the key repressors of the Blimp-1 gene (*Prdm1*). However, little is known about the activators of *Prdm1*. Candidate molecules include IRF-4 (see below), STAT3 and some of the AP-1 factors (*38*, *55*, 56).

IRF-4—IRF-4 belongs to the interferon regulatory factor (IRF) family of transcription factors (*57*). IRF-4 is expressed in both the lymphoid-lineage cells and the myeloid-lineage cells. During B-cell development, IRF-4 is expressed in the immature B cells in the bone marrow and is absent from the proliferating GC centroblasts, then it is re-expressed in a subpopulation of centrocytes in the GC and plasma cells (*58*). IRF-4 deficient mice show normal B-cell development in the bone marrow. In contrast, the maturation of later stages of the peripheral B cells is reduced, resulting in a strongly impaired immune response. The plasma cells cannot be detected in the spleen or lamina propria of *Irf-4*$^{-/-}$ mice (*59*). Recently, two groups examined in detail the function of IRF-4 in B and plasma cells. Klein *et al*. (*60*) generated mice in which *Irf-4* can be conditionally deleted in the GC B cells and the fate of the cells carrying the deletion can be monitored by the fluorescence of enhanced-GFP *in vivo*. Sciammas *et al*. (*55*), revisited the effects of germline knockout of *Irf-4*. In both studies, IRF-4 was found to be required for CSR. AID expression is strongly reduced in the IRF-4-deficient spleen B cells stimulated with LPS, or CD40 and IL-4. Because the retroviral transduction of AID partially

rescues CSR *in vitro*, IRF-4 is placed upstream of the AID expression. However, it remains unclear whether or not IRF-4 directly activates the AID gene. Klein *et al*. (*60*) proposed that the overexpression of the transcriptional repressor Id2 in the IRF-4-deficient B cells may cause the reduced AID expression, because Id2 is a negative regulator of the AID gene (*61*). In both studies, the IRF-4-deficient B cells failed to differentiate into plasma cells. Klein *et al*. (*60*) reported that Blimp-1 is induced normally but that XBP-1 is not induced in the stimulated B cells. In contrast, Sciammas *et al*. (*55*) observed a severely reduced Blimp-1 expression in stimulated B cells. Because the retroviral transduction of Blimp-1 partially rescued the IgM secretion function, which is a function of the plasma cells, the defective plasma cell differentiation is attributed to the reduced Blimp-1 expression. Indeed, IRF-4 binds to the enhancer region of the Blimp-1 gene in the activated B cells. The controversy regarding the Blimp-1 expression in the absence of IRF-4 thus requires further study.

XBP-1—XBP-1 (X-box binding protein 1) is a bZip protein in the CREB/ATF family of transcription factors (*62*). The transcriptional activity of XBP-1 is regulated in response to ER stress by the ER transmembrane endoribonuclease and kinase called IRE1. IRE1 senses the overabundance of unfolded protein in the lumen of the ER, and splices the XBP-1 mRNA, thus resulting in a transcriptionally active XBP-1 (XBP-1s) (*63*). XBP-1 directly activates the transcription of the genes encoding chaperones and enzymes which function in the ER secretory apparatus (*64*, *65*). Thus, XBP-1 is one of the key regulators of the mammalian unfolded protein response (UPR). When B cells become plasma cells, there is a huge increase in the protein load in the ER, namely in the Ig heavy and light chains. XBP-1 is critical for the execution of UPR during plasma cell differentiation. While XBP-1 is expressed from the pre-pro-B cells to the plasma cells during B-lineage development, it is expressed at the highest levels in the plasma cells (*66*). XBP-1 is essential during embryogenesis in mice. Its function in the plasma cells was revealed in the complementation of Rag2 knockout mice that lack both T and B cells with XBP-1-deficient ES cells (*67*). Because Rag proteins are essential for early immune cells to rearrange their immunoglobulin and T-cell-receptor genes as well as to continue development, B cells and T cells of the chimeras derive from XBP-1-deficient ES cells. The lymphoid chimeras deficient in XBP-1 have a profound defect in plasma cell differentiation, with few plasma cells in their periphery and severely reduced serum immunoglobulin levels. In the *XBP-1*$^{-/-}$ B cells, a large Golgi apparatus fails to develop (*66*). This may be due to a defective UPR in the absence of XBP-1. Since the B cells lacking XBP-1 express Blimp-1, XBP-1 is not upstream of Blimp-1. In contrast, the XBP-1 expression decreases in the Blimp1-deficient B cells when stimulated (*51*). The GC formation and CSR are not affected in the absence of XBP-1, thus indicating that XBP-1 is specifically required in the UPR which accompanies terminal plasma cell differentiation.

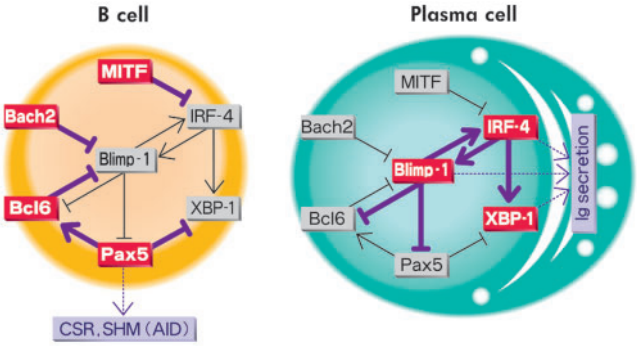

Fig. 2. **Transition of transcription factor network from B cell to plasma cells.** In B cells, Bach2, Bcl6, MITF and Pax5 repress the plasmacytic transcription factors (left). Blimp-1, IRF-4 and XBP-1 promote plasma cell differentiation (right). The red boxes indicate the genes that are expressed. The gray boxes indicate the genes that are not expressed. The bold lines and arrows indicate effective repression and effective activation, respectively. The dashed arrows represent the biological outputs. CSR, class switch recombination; SHM, somatic hypermutation; Ig, immunoglobulin.

ARCHITECTURE OF TRANSCRIPTION FACTOR NETWORKS IN B AND PLASMA CELLS

The induction of Blimp-1 appears to be a decisive event in plasma cell differentiation because Blimp-1 is not only essential but also sufficient, at least in certain contexts, for the terminal differentiation process (*7*). Starting from Blimp-1, a gene regulatory network can be assembled from the transcription factors described above (Fig. 2). The expression of Blimp-1 is kept low, if it is expressed at all, in B cells but it is induced upon terminal differentiation into plasma cells (*68*). One of the interesting features of this process is that the activities of the repressive transcription factors (*i.e*. Bach2 and Bcl6) funnel into the negative regulation of the Blimp-1 gene. The dual negative regulation of Blimp-1 is expected to ensure its silence in the B cells and the GC B cells. Because Bach2 deficiency suffices to cause the overexpression of Blimp-1 in the B cells, Bach2 and Bcl6 are not redundant to each other. The overexpression of both Blimp-1 and XBP-1 in the Bach2-deficient B cells suggest that the plasma cell program is initiated in these cells.

In the plasma cells, Blimp-1 is induced in part by IRF-4 (*55*). Because Blimp-1 is critical to induce the expression of IRF-4, these two factors may transactivate each other in order to sustain their expression once initiated (*55*). Whereas the autoregulatory loop between Blimp-1 and IRF-4 can fix the plasma cell program, Blimp-1, and IRF-4 each regulates other downstream genes to realize the plasma cell phenotype. Blimp-1 cancels the mature B-cell phenotypes by inhibiting the expression of Pax5 (*52*), which is a critical regulator of B-cell differentiation. While Blimp-1 also activates the expression of XBP-1 to deploy UPR, such activation may be indirect. IRF-4 also activates XBP-1 independently of Blimp-1 (*60*). IRF-4 also augments the expression of the immunoglobulin genes (*59*). Since Bach2 represses the 3′ LCR of the immunoglobulin heavy chain gene (*18*), the reduced levels of Bach2 in the plasma cells may also

be the cause of the higher levels of immunoglobulin gene expression. While Bcl6 is repressed by Blimp-1 in the plasma cells, it is not clear how the expression of Bach2 is silenced.

The architecture of gene networks can be classified into two categories: endogenous and exogenous (*69*). Endogenous ones are multi-stage which basically operate with an internal transcriptional program. Endogenous networks are typically found in those regulating differentiation. In this type of network, the transcription factors often regulate other transcription factor genes. In contrast, the exogenous ones constitute binary events in which the transcription factors react to external stimuli, such as stresses, and change the target gene expression. In this category, a transcription factor tends to regulate the ultimate effector genes (*e.g*. the heat shock protein genes activated by a heat shock factor in the heat shock response). The network that determines the transition of the B and plasma cells shows both characters of endogenous and exogenous gene networks. The network is exogenous in that a few key transcription factors determine the network outputs. The activity of Bach2 determines whether the network is fixed in the mature B-cell configuration or in the plasma cell configuration. The network is also exogenous in that a single transcription factor (*e.g*. Blimp-1) regulates multiple target genes. Nonetheless, the network is endogenous in that it contains multiple transcription factor-transcription factor gene pathways. Such a combination of both endogenous and exogenous network features may reflect the nature of the B-cell system in which the differentiation process and antigen reactivity of the B cells are intimately connected. This combination may make it possible to achieve two contradictory features in B cells: the capacity to stably maintain the gene network in certain configurations according to the genetic program of B-cell differentiation, and the capacity to rewire the network in response to antigens.

Several lines of evidence raise the possibility that the plasma cell differentiation program can be initiated by regulating the activity of Bach2. Phosphatidylinositol-3 (PI3) kinase regulates the responses of the B cells to antigens and T-cell stimuli (*70*). The subcellular localization of Bach2 is regulated in the B cells by the phosphorylation downstream of the PI3 kinase cascade (Fig. 1) (*26*). In addition, the subcellular localization of Bach2 is regulated by oxidative stress (*23*, *25*). It is tempting to speculate that the B-cell receptor may regulate Bach2 because it is connected with both PI3K and the production of reactive oxygen species (*26*, *71*).

While the expression of Blimp-1 determines the initiation of plasma cell differentiation, how the regulation of differentiation into the IgM-producing plasma cells or class-switched plasma cells takes place remains unclear. In the B cells, it takes a considerable length of time to carry out CSR and SHM (*72*). It thus should be critical that Blimp-1 is not induced before the completion of these events. Combined with the fact that Blimp-1 represses AID, this suggests that the timing of the Blimp-1 expression may determine whether the B cells become IgM plasma cells or other types of plasma cells. Because the Bach2-deficient B cells do not undergo CSR or SHM and express high levels of Blimp-1 (*21*), Bach2 may determine the timing of the Blimp-1 expression and thus the direction of differentiation, to switch or not to switch.

CONCLUSION

Having identified the critical transcription factors in the process of plasma cell differentiation, it is now feasible to investigate how the transition from one state (B cells) to the other (plasma cells) is regulated. Some diseases stemming from B and plasma cells, such as B-cell lymphoma and myeloma, may be caused by a deregulation of this transcription factor network. In addition, this notion is further fueled by the fact that both Bcl6 and Bach2 have previously been implicated in malignancy (*71*, *73*, *74*).

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